

**Supplementary Table 1.** Diversity of extracellularly transmitted bacterial mutualists in insects. Only those associations are listed for which the molecular identification and/or characterization of the symbionts are available, and a description of the transmission route as well as at least a putative function is reported. Exemplary references are given for each symbiosis (not exhaustive).

Insect Host		Bacterial Symbiont		Transmission Route	Biological Function(Putative) <sup>a</sup>	References
Order	Family - Genus/Species	Phylum	Genus/Species			
Hemiptera	Plataspidae - Various species	γ-Proteobacteria	'Candidatus Ishikawaella capsulata'	Capsule transmission	Nutrition <sup>a</sup>	[1-4]
	Acanthosomatidae - Various species	γ-Proteobacteria	'Candidatus Rosenkranzia clausaccus'	Egg smearing: gland secretion	Nutrition <sup>a</sup>	[5]
	Parastrachiidae - <i>Parastrachia japonensis</i>	γ-Proteobacteria	'Candidatus Benitsuchiphilus tojoi'	Egg smearing: gland secretion	Nutrition: Uric acid recycling <sup>a</sup>	[6,7]
	Pentatomidae - <i>Plautia stali</i>	γ-Proteobacteria	<i>Erwinia</i> sp.	Egg smearing: feces	Nutrition <sup>a</sup>	[8,9]
	Pyrrhocoridae - Various species	Actinobacteria	<i>Coriobacterium glomerans</i> <i>Gordonibacter</i> sp.	Egg smearing: feces	Nutrition: B vitamin supplementation	[10-13]
	Lygaeoidea and Coreoidea superfamilies - Various species	β-Proteobacteria	<i>Burkholderia</i> sp.	Environmental determination	Nutrition <sup>a</sup> , Insecticide resistance	[14-16]
	Reduviidae - <i>Rhodnius prolixus</i>	Actinobacteria	<i>Rhodococcus rhodnii</i>	Coprophagy	Nutrition: B vitamin supplementation	[17]
	Aleyrodidae - <i>Bemisia tabaci</i>	α-Proteobacteria	<i>Rickettsia</i> sp.	Environmental determination	Unknown	[18,19]
	Cicadellidae - <i>Scaphoideus titanus</i> <sup>b</sup>	α-Proteobacteria	<i>Asaia</i> sp.	Egg smearing : bacterial colonies on ovarian egg surface Environmental determination	Unknown	[20]
	Urostylididae	γ-Proteobacteria	'Candidatus Tachikawaea gelatinosa'	Jelly transmission	Unknown	[21]
Thysanoptera	Thripidae - <i>Frankliniella occidentalis</i>	γ-Proteobacteria	<i>Erwinia</i> sp.	Environmental determination	Unknown	[22,23]

Coleoptera	Chrysomelidae - <i>Macroplea mutica</i> , <i>Macroplea appendiculata</i>	γ-Proteobacteria	' <i>Candidatus Macropleicola</i> '	Egg smearing: bacterial mass droplets	Habitat colonization: secretion provision for cocoon building in wetlands	[24,25]
	Chrysomelidae - <i>Donacia marginata</i> , <i>Donacia semicuprea</i>	γ-Proteobacteria	Unspecified	Egg smearing: bacterial mass droplets	Habitat colonization: secretion provision for cocoon building in wetlands	[25]
	Staphylinidae - <i>Paederus sabaeus</i>	β-Proteobacteria	<i>Pseudomonas</i> sp.	Egg smearing : unspecified	Defense: biosynthesis of defensive toxin	[26,27]
Diptera	Tephritidae - <i>Bactrocera oleae</i>	γ-Proteobacteria	' <i>Candidatus Erwinia dacicola</i> '	Egg smearing: gland secretion	Nutrition <sup>a</sup> : protein hydrolysis and amino acid provision	[28,29]
	Tephritidae - <i>Bactrocera dorsalis</i>	γ-Proteobacteria	<i>Klebsiella oxytoca</i>	Egg smearing: gland secretion	Reproductive behavior: adult attractant	[30]
	Tephritidae - <i>Ceratitis capitata</i>	γ-Proteobacteria	<i>Klebsiella oxytoca</i>  <i>Pectobacterium cypripedii</i>	Egg smearing: biofilm on egg surface, potential fecal contamination	Nutrition: pectin degradation and nitrogen metabolism.  Reproductive behavior: shortened mating latency in males	[31,32]
	Muscidae - <i>Musca domestica</i>	γ-Proteobacteria, Firmicutes	<i>Klebsiella oxytoca</i>  <i>Bacillus cereus</i>	Egg smearing : unspecified	Defense: against competing fungi in substrate for larval nutrition.  Nutrition: diet supplement for larvae  Reproductive behavior: influence on oviposition decisions.	[33]

<b>Diptera</b> (Cont'd)	Culicidae - <i>Anopheles</i> spp	$\alpha$ -Proteobacteria	<i>Asaia</i> sp.	Egg smearing: bacterial colonies on ovarian egg surface Environmental determination	Nutrition, defense, mediation of gut homeostasis and microbial equilibrium <sup>a</sup>	[34-38]
	Culicidae - <i>Aedes aegypti</i>	$\alpha$ -Proteobacteria	<i>Asaia</i> sp.	Egg smearing: unspecified Environmental determination	Nutrition, defense, mediation of gut homeostasis and microbial equilibrium <sup>a</sup>	[36]
<b>Hymenoptera</b>	Crabronidae - <i>Philanthus</i> spp., <i>Trachypus</i> spp., <i>Philanthinus</i> spp.	Actinobacteria	' <i>Candidatus Streptomyces philanthi</i> '	Brood cell contamination: antennal gland secretion	Defense: Protection against detrimental fungi	[39-42]
	Formicidae - Various Attine species	Actinobacteria	<i>Pseudonocardia</i> sp.	Social acquisition	Defense: production of antifungal compound against cultivar pathogen ( <i>Escovopsis</i> sp.)	[43-45]
	Apidae - <i>Apis</i> spp.	$\alpha$ -, $\beta$ -, and $\gamma$ -Proteobacteria, Firmicutes	<i>Gilliamella apicola</i> , <i>Snodgrassella alvi</i>  <i>Lactobacillus</i> sp., <i>Bifidobacterium</i> sp.  and unspecified Acetobacteraceae	Social acquisition	Pectin degradation <sup>a</sup>	[46-50]
	Apidae - <i>Bombus</i> spp.	$\gamma$ , $\beta$ -Proteobacteria	<i>Burkholderia</i>  <i>Gilliamella apicola</i> , <i>Snodgrassella alvi</i>	Social acquisition: coprophagy	Defense: protection against parasites	[46,51]
<b>Blattaria</b>	Blattidae - <i>Shelfordella lateralis</i>	Firmicutes, Bacteroidetes, $\delta$ - Proteobacteria	<i>Clostridium</i> sp., <i>Succinispira</i> sp.  <i>Enterococcus</i> sp., <i>Erysipelothrix</i> sp.,	Coprophagy	Nutrition <sup>a</sup>	[52]

<b>Blattaria (Cont'd)</b>	Polyphagidae - <i>Cryptocercus punctulatus</i>	Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Spirochaetes	<i>Treponema</i> sp., <i>Spirochaeta</i> sp., others unspecified	Coprophagy	Nutrition <sup>a</sup>	[53]
<b>Isoptera</b>	Rhinotermitidae - <i>Reticulitermes</i> spp. Kalotermitidae - <i>Cryptotermes</i> spp. <i>Neotermes castaneus</i> Termopsidae - <i>Zootermopsis angusticollis</i> (lower termites)	Spirochaetes, Firmicutes, Bacteroidetes, Proteobacteria	<i>Treponema</i> sp. <i>Clostridiales</i> spp. <i>Lactococcus</i> sp. <i>Enterococcus</i> sp. <i>Bacteroides</i> spp. <i>Desulfovibrio</i> sp.	Social acquisition	Nutrition: N <sub>2</sub> fixation, acetogenesis, nitrogen recycling, cellulolytic activity.	[54-56]
	Termitidae - <i>Microcerotermes</i> spp. <i>Nasutitermes</i> spp. (higher termites)	Spirochaetes, Firmicutes, Bacteroidetes, Fibrobacteres.	<i>Treponema</i> sp., <i>Clostridiales</i> sp. <i>Bacteroidales</i> sp.,	Social acquisition, Environmental determination	Nutrition: N <sub>2</sub> fixation, acetogenesis, nitrogen recycling, cellulolytic activity	[57-59]
<b>Orthoptera</b>	Acrididae - <i>Schistocerca gregaria</i>	γ-Proteobacteria	<i>Pantoea</i> , <i>Enterococcus</i> , <i>Serratia</i> , <i>Klebsiella</i> , <i>Acinetobacter</i>	Environmental determination	Unknown	[60,61]

<sup>a</sup> No conclusive evidence, but hypotheses or suggestive results are reported.

**Supplementary Table 2.** Genome size and GC content for intra- and extracellularly transmitted bacterial symbionts in insects (as illustrated in Figure 3).

Symbiont	Symbiont Localization	Symbiont Transmission Route	Genome Size (Mb)	GC content (in %)	Reference
' <i>Candidatus Zinderia insecticola</i> '	Intracellular	Intracellular	0.21	13.5	[62]
' <i>Candidatus Sulcia muelleri GWSS</i> '	Intracellular	Intracellular	0.25	22.4	[63]
' <i>Candidatus Uzinura diaspodicola</i> '	Intracellular	Intracellular	0.26	30.2	[64]
' <i>Candidatus Moranella endobia</i> '	Intracellular	Intracellular	0.54	43.5	[65]
' <i>Candidatus Blattabacterium sp.</i> '	Intracellular	Intracellular	0.59	27.5	[66]
<i>Buchnera aphidicola</i>	Intracellular	Intracellular	0.66	26.4	[67]
<i>Baumannia cicadellinicola</i>	Intracellular	Intracellular	0.69	33.2	[68]
' <i>Candidatus Tachikawaea gelatinosa</i> '	Extracellular	Extracellular (Jelly transmission)	0.70	37.5	[21]
' <i>Candidatus Ishikawaella capsulata</i> '	Extracellular	Extracellular (Capsule transmission)	0.75	38.5	[4]
' <i>Candidatus Rosenkranzia clausaccus</i> '	Extracellular	Extracellular (Egg smearing)	0.94	37.6	[5]
Gut symbiont of <i>Adomerus triguttulus</i>	Extracellular	Extracellular (Egg smearing)	1.22	53.6	[7]
<i>Coriobacterium glomerans</i>	Extracellular	Extracellular (Egg smearing)	2.11	60.4	[69]

<i>Treponema azonutricum</i>	Extracellular	Extracellular (Social acquisition)	3.91	50	[56]	
<i>Gilliamella apicola</i>	Extracellular	Extracellular (Social acquisition)	2.26	35	[70]	
<i>Snodgrassella alvi</i>	Extracellular	Extracellular (Social acquisition)	2.3	43	[70]	
<i>Burkholderia</i> sp. strain RPE64	Extracellular	Extracellular (Environmental determination)	6.96	63.5	[71]	

**Supplementary Table 3.** Non-exhaustive list of intracellularly transmitted symbionts in insects.

Symbiont	Host (Order: Family)	Reference
' <i>Candidatus Zinderia insecticola</i> '	Spittlebugs Hemiptera: Clastopteridae	[62]
' <i>Candidatus Sulcia muelleri GWSS</i> '	Spittlebugs Hemiptera: Clastopteridae	[63]
' <i>Candidatus Uzinura diaspadicola</i> '	Scale insects Hemiptera: Diaspididae	[64]
' <i>Candidatus Moranella endobia</i> '	Mealybugs Hemiptera: Pseudococcidae	[65]
<i>Buchnera aphidicola</i>	Aphids Hemiptera: Aphididae	[67]
<i>Baumannia cicadellinicola</i>	Sharpshooters Hemiptera: Homalodisca	[68]
' <i>Candidatus Portiera aleurodidarum</i> '	Whiteflies Hemiptera: Sternorrhyncha	[72]
' <i>Candidatus Hodgkinia cicadicola</i> '	Cicadas Hemiptera: Cicadidae	[73]
<i>Wolbachia</i> Spp.	Bed bugs Hemiptera: Cimicidae	[74]

<i>'Candidatus Cardinium hertigii'</i>	Midges Diptera: Ceratopogonidae	[75]
<i>'Candidatus Blochmannia floridanus'</i>	Carpenter ants Hymenoptera: Formicidae	[76]
<i>'Candidatus Blattabacterium cuenoti'</i>	Termites Isoptera: Mastotermitidae	[77]
<i>Nardonella spp</i>	Weevils Coleoptera: Curculionidae	[78]
<i>'Candidatus Blattabacterium sp.'</i>	Cockroaches Blattodea: Dictyoptera	[66]

**Supplementary Table 4.** Evolutionary implications of symbiont transmission routes in insects

Insect Host (Order: Family)	Bacterial Symbiont	Symbiont Localization	Extracellular Transmission Route	Maternal Provisioning of Symbionts	Specialized Structures for Symbiont Cultivation and/or Transmission	Strict Host Symbiont Co-cladogenesis	Symbiont Genome Erosion	References
<i>Urostylis</i> spp. (Hemiptera: Urostylidae)	' <i>Candidatus Tachikawaea gelatinosa</i> '	Midgut crypts	Jelly transmission	+	+	+	+	[21]
<i>Megacopta</i> spp. (Hemiptera: Plataspidae)	' <i>Candidatus Ishikawaella capsulata</i> '	Midgut crypts	Symbiont capsule	+	+	+	+	[3,4]
<i>Elasmostethus</i> spp. (Hemiptera: Acanthosomatidae)	γ-Proteobacteria	Midgut crypts	Egg smearing	+	+	+	+	[5]
<i>Philanthus</i> spp. (Hymenoptera: Crabronidae)	<i>Streptomyces philanthi</i>	Antennal gland reservoirs	Brood cell smearing	+	+	+/-	+/-	[79,80]
<i>Pyrrhocoris apterus</i> (Hemiptera: Pyrrhocoridae)	<i>Coriobacterium glomerans</i>	Midgut lumen	Egg smearing	+	-	-	-	[10,69]
<i>Plautia</i> spp. (Hemiptera: Pentatomidae)	<i>Erwinia</i> spp.	Midgut crypts	Egg smearing	+	-	-	Unexamined	[8,9]
<i>Apis</i> spp. and <i>Bombus</i> spp. (Hymenoptera: Apidae)	<i>Snodgrassella alvi</i> , <i>Gilliamella apicola</i>	Midgut lumen	Social transmission	+	-	-	+/-	[49,70]
<i>Riptortus pedestris</i> (Hemiptera: Alydidae)	<i>Burkholderia</i> spp.	Midgut crypts	Environmental uptake	-	-	-	-	[15,71,81]

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